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INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)

Applicant's or agent's file reference P31003WONCB	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/GB 03/03536	International filing date (day/month/year) 13.08.2003	Priority date (day/month/year) 14.08.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant ROSLIN INSTITUTE (EDINBURGH) et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
 - ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 11.03.2004	Date of completion of this report 14.09.2004
Name and mailing address of the International preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Favre, N Telephone No. +49 89 2399-7363 

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International application No. PCT/GB 03/03536

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-29 as originally filed

Claims, Numbers

1-22 filed with telefax on 27.07.2004

Drawings, Sheets

1/17-17/17 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-22
	No: Claims	
Inventive step (IS)	Yes: Claims	1-14
	No: Claims	15-22
Industrial applicability (IA)	Yes: Claims	1-22
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

1. Sequence listing pages 1-15 filed with the letter of 14.11.2003 do not form part of the application (Rule 13^{ter}.1(f) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (Molecular Biology of the Cell, 2000, 11:3645-3660) discloses (cf. abstract, figures 1 and 4-7) a method of determination of the sex of an avian subject from which the subject-matter of independent claim 1 differs in that it refers to the use of nucleic acid probes based on a different sequence (FAF-4 as shown in Figure 11).
 - 1.1 The problem to be solved by independent claim 1 appears therefore to be the provision of probes based on an alternative genetic marker for the determination of the sex of an avian subject.
 - 1.2 Such alternative genetic markers for the determination of the sex of an avian subject and methods for their identification/isolation were already known in the art, see for instance D2 (WO-A-97 49806; e.g. abstract and claims), D3 (US-A-5 707 809; e.g. abstract and claims), D4 (WO-A-96 39505; e.g. abstract and claims), D5 (British Poultry Science, 2001, 42:134-138; e.g. "Material and Methods" and figures 1-5), D6 (Molecular Ecology, 1998, 7:1071-1075; e.g. "Material and methods" and figure 1), D7 (Journal of Avian Biology, 1999, 30:116-121; e.g. abstract and figure 1) or D8 (Chromosome Research, 1997, 5:93:101; e.g. abstract and figure 4).

In response to arguments considering that the identification/isolation of the nucleic acid sequence of figure 11 (FAF-4) of the present application only required routine experimentation from the skilled person, the applicant has counter-argued that all the markers taught in the prior art have variants (or homologues in the case of

genes) on the male Z-chromosome, whereas FAF-4 surprisingly has not, hence providing an improved tool for avian sex determination.

It is therefore to be considered that these findings, i.e. lack of a counterpart on the male chromosome, could not have been expected and have thus to be considered as surprising.

- 1.3 The problem to be solved by the present invention may therefore be regarded as the provision of an improved method based on probes based on a better marker for the determination of the sex of an avian subject.
- 1.4 Since none of the prior art documents at hand disclose or fairly suggest the nucleic acid FAF-4, and thus do not suggest its beneficial use as a marker for the determination of the sex of an avian subject, the solution proposed in independent claim 1 is considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.
- 1.5 Dependent claims 2-11 further define specific embodiments of the novel and inventive method of claim 1.
Dependent claims 2-11 are hence also considered to meet the requirements of Articles 33(2) and 33(3) PCT.
- 1.6 The subject-matter of independent claim 12 does not differ from the subject-matter of independent claim 1 (lack of conciseness in the sense of Article 6 PCT), since it refers to the same technical features.
The subject-matter of independent claim 12 is thus also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.
- 1.7 The subject-matter of independent claim 13 is an isolated nucleic acid molecule having a sequence as shown figure 11, i.e. FAF-4.
In view of the arguments presented herein-above for the subject-matter of independent claim 1, the subject-matter of independent claim 13 is also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.
- 1.8 A similar argumentation also applies for the subject-matter of independent claim 14 which refers to a kit comprising such sequences.
The subject-matter of independent claim 14 is hence considered to meet the requirements of Articles 33(2) and 33(3) PCT.

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2. However, the subject-matter of claims 15-22 refers to polypeptides and antibodies thereagainst which polypeptides could be coded by one of the reading frame of the nucleic acid sequences of figure 11.

In view of the arguments presented herein-above, it appears that the technical problem solved by the subject-matter of these claims would be the provision of improved means to determine the sex of an avian subject.

There is however no indication or experimental result in the application as filed that would demonstrate that this problem has effectively been solved by the subject-matter of these claims, i.e. the polypeptides that are potentially/theoretically coded by the nucleic acid sequences of figure 11.

Since these claims do not appear to solve a technical problem, their subject-matter cannot be considered to be inventive in the sense of Article 33(3) PCT.

CLAIMS

1. A method for the determination of the sex of an avian subject, the method comprising contacting a sample from said subject with a nucleic acid probe comprising an at least 6 base pair fragment from a target nucleic acid sequence FAF-4 as shown in Figure 11, or a sequence complementary or homologous thereto.
2. A method as claimed in claim 1, in which the nucleic acid probe comprises a probe sequence of at least 15 nucleotides.
3. A method as claimed in claim 1 or claim 2, in which the nucleic acid probe is sequence FAF-4 as shown in Figures 11 or a fragment thereof.
4. A method as claimed in any one of claims 1 to 3, in which the avian is a member of Class Aves.
5. A method as claimed in claim 4, in which the avian is selected from the group consisting of *Gallus gallus* (chicken), turkey, quail and guinea fowl.
6. A method as claimed in any one of claims 1 to 5, in which the sample is allantoic fluid or amniotic fluid.
7. A method as claimed in any preceding claim, in which the sample is taken from an egg.
8. A method as claimed in any preceding claim, in which the analysis of the sample comprises a nucleic acid amplification procedure
9. A method as claimed in claim 8, in which the nucleic acid amplification procedure is exponential amplification of the target sequence.

10. A method as claimed in claim 9, in which the nucleic acid amplification procedure is linear amplification of the target sequence.

5 11. A method as claimed in claim 10, which comprises amplification of RNA in the sample.

12. The use of a nucleic acid sequence or a fragment thereof according to any one of Figure 11 in a method according to any one of claims 1 to 11.

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13. An isolated nucleic acid molecule as shown in Figure 11.

14. A kit of parts comprising a nucleic acid probe comprising an at least 6 base pair fragment from an isolated nucleic acid molecule FAF-4 as shown in Figure 11 for determining the sex of an avian subject, or a sequence complementary or homologous thereto.

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15. A polypeptide or fragment thereof coded for by a nucleic acid sequence one of Figure 11.

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16. A polypeptide comprising a sequence as shown in Figure 15(d).

17. A vector comprising a nucleic acid sequence of Figure 11.

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18. A host cell comprising a vector as defined in claim 17.

19. An antibody to a polypeptide as defined in claim 15 or claim 16.

20. An antibody as claimed in claim 19 which is a monoclonal antibody.

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21. A method for the determination of the sex of an avian subject, the method comprising contacting a sample from said subject with an antibody to a polypeptide as defined in claim 15 or claim 16.
- 5 22. A kit of parts comprising an antibody as defined in claim 19 or claim 20 for determining the sex of an avian subject.